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## Comparison of experimental models for *Streptococcus suis* infection of conventional pigs

Francisco J. Pallarés, Patrick G. Halbur, Cameron S. Schmitt, James A. Roth, Tanja Opriessnig, Peter J. Thomas, Joann M. Kinyon, Dee Murphy, Dagmar E. Frank, Lorraine J. Hoffman

### Abstract

Four different experimental models for *Streptococcus suis*-induced disease were compared to find a model that closely mimics naturally occurring disease in conventional pigs. Fourteen, 2-week old pigs free of *S. suis* type 2 were used in 2 experiments. In experiment 1, 3 pigs were inoculated intravenously (IV) and 3 pigs intranasally (IN) with *S. suis*. Two out of 3 of the IV-inoculated pigs exhibited signs of severe central nervous system disease (CNS) and were euthanized. *Streptococcus suis* type 2 was isolated from whole blood, joints, and serosal surfaces of both pigs. No clinical signs and no growth of *S. suis* were detected in the IN-inoculated pigs. In experiment 2, 4 pigs were inoculated IV and another 4 were inoculated IN with the same isolate as in experiment 1. One hour before inoculation the IN-inoculated pigs were given 5 mL of 1% acetic acid intranasally (IN-AA). All the IV-inoculated pigs showed CNS disease and lameness, and 2 of the pigs became severely affected and were euthanized. All the IN-AA inoculated pigs exhibited roughened hair coats and 2 pigs developed severe CNS disease and were euthanized. *Streptococcus suis* was isolated from the joints and blood of 3 pigs in the IV-inoculated group. *Streptococcus suis* was isolated from blood of 2 pigs, meninges of 3 pigs, and joints of 1 pig in the IN-AA inoculated group. Natural exposure to *S. suis* most likely occurs by the intranasal route. The IN-AA model should serve as a good model for *S. suis*-induced disease, because the natural route of exposure is intranasal and the IN-AA model was effective in inducing disease that mimics what is observed in the field.

### Résumé

Quatre modèles expérimentaux différents d'infections causées par *Streptococcus suis* ont été comparés afin de trouver le modèle qui imiterait le mieux une infection naturelle chez des porcs élevés de manière conventionnelle. Quatorze porcelets âgés de 2 semaines et exempts de *S. suis* sérotype 2 ont été utilisés dans deux expériences. Dans la première expérience, 3 porcs furent inoculés par voie intra-veineuse (IV) et 3 par voie intra-nasale (IN) avec *S. suis*. Deux des 3 porcs inoculés IV montrèrent des signes d'atteinte sévère du système nerveux central (SNC) et ont été euthanasiés. *Streptococcus suis* sérotype 2 a été isolé à partir du sang entier, des articulations et des séreuses des deux porcs. Aucune signe clinique et aucune croissance de *S. suis* ne fut détecté à partir des animaux inoculés IN. Lors de la deuxième expérience, 4 porcs furent inoculés IV et 4 autres IN à l'aide du même isolat que lors de l'expérience 1. Chez les porcs inoculés IN, 1 h avant l'inoculation on leur administra 5 mL d'acide acétique 1% par voie intra-nasale (IN-AA). Tous les porcs inoculés IV montrèrent des signes d'atteinte du SNC et de boiterie, et 2 porcs ont été euthanasiés à cause de la sévérité des signes présentés. Tous les animaux IN-AA avaient mauvais poil et 2 porcs développèrent une atteinte sévère du SNC et ont été euthanasiés. *Streptococcus suis* fut isolé des articulations et du sang de 3 porcs du groupe inoculé IV. Parmi les animaux IN-AA on isola *S. suis* du sang de 2 porcs, des méninges de 3 porcs et des articulation de 1 porc. L'exposition naturelle à *S. suis* se produit fort probablement par la voie IN. Le modèle expérimental IN-AA devrait servir de bon modèle pour les maladies associées à *S. suis* étant donné que la voie naturelle d'exposition est intra-nasale et que le modèle IN-AA était efficace à induire une pathologie qui imitait bien ce qui est observé dans les élevages.

(Traduit par Dr Serge Messier)

*Streptococcus suis* is a common pathogen in swine farms worldwide and has been associated with a variety of diseases such as meningitis, arthritis, bronchopneumonia, and septicemia in young pigs (1). *Streptococcus suis* is transferred from vaginal secretions to the oral cavity of the piglet during parturition (2) and colonizes the tonsil soon after birth (3). The mechanism of systemic spread following

oropharynx exposure is not well understood. It is likely that the bacteria reaches the cerebrospinal fluid (CSF) compartment, joint spaces, and serosal cavities by traveling within monocytes (4). Pneumonia may result from inhalation or from bacteremia, either by direct infection of the alveoli or by transport of the bacteria in monocytes to the alveoli (5).

Veterinary Diagnostic Laboratory (Pallarés, Halbur, Opriessnig, Thomas, Kinyon, Murphy, Hoffman); Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50011, USA (Schmitt, Roth, Frank); and Histología y Anatomía Patológica, Facultad de Veterinaria, Universidad de Murcia, 30071 Murcia, Spain (Pallarés).

Address all correspondence and reprint requests to Dr. Patrick G. Halbur; telephone: (515) 294-1950; fax: (515) 294-6961; e-mail: pghalbur@iastate.edu

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Excessive temperature fluctuation, high relative humidity, overcrowding, and an age spread of more than 2 wk between pigs in the same room are commonly associated with high percentages of *S. suis* carrier animals in the nursery (6). Susceptibility to *S. suis* infection can be increased by concurrent infection by viruses such as porcine reproductive and respiratory syndrome virus (PRRSV) (7,8) or pseudorabies virus (PRV) (9).

Intravenous (IV) inoculation of *S. suis* results in high morbidity and mortality (10,11). In contrast, intranasal (IN) *S. suis* inoculation (12–14) often results in no or limited disease, lesions, and mortality. The natural route of exposure to *S. suis* is thought to be oronasal, thus, an oral or intranasal model is desirable for pathogenesis studies and vaccine efficacy trials. Acetic acid (AA) has been used for its effect as an irritant of the upper respiratory tract (15) and as a predisposer to experimental infections with toxigenic *Pasteurella multocida* (16) or *S. suis* (17). The objectives of this study were to compare 4 different experimental models for *S. suis* induced disease and select the model that closely mimics naturally occurring disease in conventional pigs.

The study was approved by the Iowa State University Committee on Animal Care and Use. Fourteen specific pathogen free (SPF) pigs from a sow unit free of PRRSV, *Mycoplasma hyopneumoniae*, and transmissible gastroenteritis virus based on periodic serologic monitoring of the breeding herd, were used in 2 different experiments. The pigs were weaned at 12 d of age and transported to the Iowa State University Livestock Infectious Disease Isolation Facility. The experimental pigs were determined to be free of *S. suis* type 2, *Bordetella bronchiseptica*, and *Haemophilus parasuis* by pre-inoculation culture of tonsil and nasal swabs.

In experiment 1, 6 pigs were randomly divided into 2 groups of 3 pigs and housed in 2 different rooms in 1.2 × 2.4 m<sup>2</sup> nursery decks and fed *ad libitum*. The air flow into the rooms was 10 to 15 air changes per hour. One group was inoculated IV and the other IN, both with 2 mL of 1.185 × 10<sup>9</sup> colony forming units (CFU)/mL *S. suis* serotype 2, prepared as previously described (18). Room temperature was decreased from 26.5°C to 15.5°C 12 h before the inoculation to generate an environmental stress situation in the pigs inoculated IN. The room temperature was changed (15.5°C to 26.5°C and back to 15.5°C) every 4 h and a fan was placed at one end of the pen to create a continuous draft from 2.2 to 10 m/s at pig level for the duration of the study in the room housing the IN group. Blood samples for serology and whole blood for bacteriology were collected from the jugular vein using a single-use blood collection system (Vacutainer; Becton Dickinson, Franklin Lakes, New Jersey, USA) and a single-use blood collection system containing EDTA (Vacutainer; Becton Dickinson), respectively, prior to inoculation.

In experiment 2, 8 pigs were randomly divided into 2 groups of 4 pigs and housed in 2 different rooms in 1.2 × 2.4 m<sup>2</sup> nursery decks and fed *ad libitum*. The air flow into the rooms was 10 to 15 air changes per hour and the temperature was 26.5°C. One group was inoculated IV and the other IN, both with 2 mL of 1.23 × 10<sup>9</sup> CFU/mL of the same isolate as used in experiment 1. One hour before inoculation, IN inoculated pigs were administered 5 mL of 1% AA (pH 3.3) IN (IN-AA) as an irritant (15) to predispose the animals to *S. suis* infection. Whole blood and sera were collected for bacteriology and serology prior to inoculation, as described in

experiment 1. Whole blood was also collected in EDTA tubes at 0, 1, 3, and 5 d postinoculation (DPI) for bacteriology.

In both experiments, animals were monitored twice a day and rectal temperatures were taken every 12 h. Pigs exhibiting severe central nervous system (CNS) disease (ataxia, postration, or opisthotonus) or severe joint swelling and lameness resulting in recumbency were immediately euthanized by electrocution and exsanguination, and necropsied. Complete necropsies were performed on all remaining pigs on day 7 in experiment 1 and on day 8 in experiment 2. Blood, meningeal swabs, serosa swabs, and joint swabs were collected for bacteriology. Brain, cerebellum, lung, joint tissue, spleen, and liver were collected in 10% neutral buffered formalin for histopathology.

All samples for bacteriology were streaked onto sheep blood agar plates. They were incubated at 37°C in 5% CO<sub>2</sub> for 72 h. Alpha-hemolytic streptococcus-like colonies were tested for growth in 6.5% NaCl and production of amylase (19). Representative colonies that did not grow in NaCl and were positive for production of amylase were serotyped using the coagglutination procedure to determine if they were *S. suis* type 2 (20).

In experiment 1, 2 pigs in the IV-inoculated group exhibited severe CNS signs, joint swelling, and high fever (> 40°C) by 1 DPI and were euthanized. No clinical signs or fever were observed in the other pig of this group or the 3 pigs of IN-inoculated group. Both IV-inoculated pigs necropsied at 1 DPI had fibrinous tags on the liver and intestine, and excessive joint fluid. No gross lesions were observed in other tissues. The other pig of this group and the 3 pigs in the IN-inoculated group were necropsied at 7 DPI. No gross lesions were observed. Microscopically, both IV-inoculated pigs euthanized at 1 DPI had moderate fibrinosuppurative and lymphohistiocytic synovitis. No microscopic lesions were observed in the other pig of this group or the 3 IN-inoculated pigs. Cultures of preinoculation whole blood were negative for *S. suis*. *Streptococcus suis* type 2 was isolated from whole blood, joints, and serosal surfaces in both pigs of the IV-inoculated group euthanized at 1 DPI, and low numbers of *S. suis* were isolated from joints of the 3rd pig of this group at necropsy on 7 DPI. No growth of *S. suis* type 2 was detected in blood, joints, or serosa surfaces of any of the IN-inoculated pigs (Table I).

In experiment 2, all pigs in the IV-inoculated group had slight to moderate CNS signs (ataxia) and lameness beginning by 1 DPI. Two pigs had severely swollen joints and moderate to severe CNS signs (ataxia, recumbency, opisthotonus). Two severely affected pigs had markedly elevated temperatures (> 41°C) the day they were euthanized. All pigs in the IN-AA inoculated group exhibited roughened hair coats at 2 DPI. Two pigs were lethargic, exhibited severe CNS signs, and had fevers (> 41°C) at 4 DPI. They were euthanized at that time. Two pigs in the IV-inoculated group had tags of fibrin on the liver and intestine, and 3 had increased joint fluid. Two pigs in the IN-AA inoculated group had increased joint fluid and fibrinous tags on the liver and intestines, and 2 had red-purple areas of consolidation in the right caudal lung lobe affecting approximately 5 to 15% of the lung tissue. Microscopic examination of tissues from the IV-inoculated pigs revealed that 2 had mild multifocal suppurative interstitial pneumonia, and 3 had mild to severe fibrinosuppurative and lymphohistiocytic synovitis. Examination of tissues from the IN-AA group revealed

**Table I. Presence/absence (+/–) of *S. suis* type 2 in blood at 0, 1, 3, and 5 d postinoculation (DPI); and in blood, brain, serosal surfaces, and joints at the time of necropsy**

Experiment	Group	Pig	Necropsy (DPI)	Blood collected prior to necropsy at DPI				Samples collected at necropsy			
				0	1	3	5	Blood	Brain	Serosal surfaces	Joints
1	IV	#1	1	–	NT	NT	NT	+	–	+	+
	IV	#2	7	–	NT	NT	NT	–	–	–	+
	IV	#3	1	–	NT	NT	NT	+	–	+	+
	IN	#4	7	–	NT	NT	NT	–	–	–	–
	IN	#5	7	–	NT	NT	NT	–	–	–	–
	IN	#6	7	–	NT	NT	NT	–	–	–	–
2	IV	#1	8	–	+	+	+	–	–	–	+
	IV	#2	6	–	+	+	+	+	–	–	–
	IV	#3	8	–	+	+	+	+	–	–	–
	IV	#4	5	–	+	+	+	+	–	–	+
	IN-AA	#5	4	–	–	–	NT	+	+	–	+
	IN-AA	#6	8	–	+	+	–	–	+	–	–
	IN-AA	#7	4	–	+	–	NT	+	+	–	–
	IN-AA	#8	8	–	–	–	–	–	–	–	–

IV: intravenous injection

IN: intranasal injection

IN-AA: intranasal acetic acid injection

+: positive

–: negative

NT: Not taken

that 2 had severe fibrinosuppurative meningitis, and 4 had mild multifocal suppurative interstitial pneumonia. Table I shows the presence /absence (+/–) of *S. suis* type 2 in blood at 0, 1, 3, and 5 DPI; and in blood, brain, serosal surfaces, and joints after necropsy. At the time of the necropsy, *S. suis* type 2 was isolated from blood of 3 pigs and joints of 2 pigs in the IV-inoculated group. In the IN-AA inoculated group, *S. suis* type 2 was isolated from the blood of 2 pigs, meninges of 3 pigs, and joints of 1 pig at necropsy. No growth of *S. suis* type 2 was detected in any tissue or blood of 1 pig in the IN-AA group.

Intravenous inoculation models have been used for studying *S. suis* pathogenesis and testing vaccine efficacy (4,10,11); however, oronasal spread is thought to be the natural route of *S. suis* exposure and transmission. In previous experimental IN models of *S. suis* inoculation, the percentage of pigs exhibiting clinical signs of *S. suis*-associated disease was very low. In one study, clinical disease of pigs inoculated IN with *S. suis* was reported in 2/14 pigs and mortality was 1/14 (13). In another study, clinical signs were reported in 1/4 pigs infected with a virulent strain of *S. suis*, and 0/3 pigs when a low virulent strain of *S. suis* was used (12). In another experiment where IN inoculation was used (14), clinical signs were not found in pigs (0/10) experimentally infected with *S. suis*. Higher morbidity and mortality associated with *S. suis* can be reproduced using co-infection with other pathogenic agents, such as PRRSV (8,14,18,21), *Bordetella bronchiseptica* (12), or PRV (9).

In experiment 1, the use of IN *S. suis* inoculation without pretreatment did not cause any clinical disease and *S. suis* could

not be recovered from any tissue. Intranasal inoculation of *S. suis* and application of environmental stressors (wide room temperature fluctuation and air drafts) were not sufficient to induce *S. suis*-associated disease. In experiment 2, pretreatment of 2-week-old SPF pigs with AA followed by IN inoculation of *S. suis* resulted in the development of clinical signs typical of *S. suis* in 4/4 pigs and mortality in 2/4 pigs. Similar results were recently described by Bak et al (22) where the mortality was 4/7 using IN-AA prior to IN inoculation of *S. suis* in 7-week-old SPF pigs. Bak et al (17) also induced a high mortality rate (11/12) in 6- to 8-week-old SPF pigs, that were aerosolized with *S. suis* following IN-AA drip. Clinical signs were not seen when both the AA and *S. suis* were aerosolized in 6 to 7-week-old minipigs (23). It appears that IN drip rather than aerosolization of the AA is important to predispose the pigs to *S. suis* septicemia.

Brown (24) induced *S. suis*-associated clinical signs and mortality in 5 out of 6, 4-week-old conventional pigs inoculated with *S. suis* following aerosolized exposure to ammonium hydroxide. The *S. suis* isolate used in that study was the same isolate and a similar dose to what was used in this study. Our IN-AA inoculation is simple, efficient and requires no special safety precautions; facilities; or equipment, such as air chambers or nebulizers, needed for the ammonium hydroxide or AA aerosolized models.

The results of this experiment suggest that the mucosal damage, stress, or both induced by the IN-AA allows *S. suis* to become septicemic and induce clinical signs and lesions that are similar to those observed in field cases of *S. suis*. The most common clinical

signs observed in field cases of *S. suis*-associated disease are CNS signs, lameness, and pneumonia in nursery pigs. These clinical signs and lesions were reproduced in the IN-AA model described herein. In fact, pigs in the IN-AA inoculated group were the only ones where *S. suis* was isolated from meninges. *Streptococcus suis* infection is thought to be contracted by inhalation and, thus, the IN-AA model may be better than the IV model to study the pathogenesis of *S. suis*-associated disease and to test vaccine efficacy. However, low numbers of pigs were used in this study and results may vary with the immunological status and, perhaps, with the genetic susceptibility of the pigs utilized in this model.

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